

IN THE CLAIMS

1-28 (Cancelled)

29. (Previously Presented) A method of detecting an endocrine disrupting action of a test substance, comprising:

- (A) culturing a cell that is sensitive to an endocrine hormone in the presence of the endocrine hormone and the test substance and detecting a gene expression pattern (1) of said cell; and
- (B) culturing said cell that is sensitive to an endocrine hormone in the presence of the endocrine hormone, but in the absence of the test substance, and detecting a gene expression pattern (2) of said cell; and/or
- (C) culturing said cell that is sensitive to an endocrine hormone in the absence of the endocrine hormone, but in the presence of the test substance, and detecting a gene expression pattern (3) of said cell; and/or
- (D) culturing said cell that is sensitive to an endocrine hormone in the absence of the endocrine hormone and in the absence of the test substance, and detecting a gene expression pattern (4) of said cell; and
- (E) comparing gene expression pattern (1) with gene expression pattern (2) and/or (3) and/or (4) to determine the endocrine disrupting activity of the test substance, wherein the increased or decreased expression of a gene in expression pattern (1) compared to the same gene in expression pattern (2) and/or (3) and/or (4) is indicative of endocrine disrupting action by the test substance.

30. (Previously Presented) The method of Claim 29, wherein gene expression pattern (1) is compared with gene expression pattern (2).

31. (Previously Presented) The method of Claim 29, wherein gene expression pattern (1) is compared with gene expression pattern (3).

32. (Previously Presented) The method of Claim 29, wherein gene expression pattern (1) is compared with gene expression pattern (2) and gene expression pattern (3).

33. (Previously Presented) The method of Claim 29, comprising comparing gene expression pattern (1) with gene expression pattern (4).

34. (Previously Presented) The method of Claim 29, wherein said gene expression patterns are measured by determining the variation in the amount of gene transcription.

35. (Previously Presented) The method of Claim 29, comprising recovering RNA corresponding to each gene expression pattern, optionally producing cDNA corresponding to said RNA, and comparing said RNA or cDNA of (A) with that of (B) and/or (C) and/or (D) to determine the endocrine disrupting activity of the test substance, wherein a difference in the amount of RNA or cDNA between (A) and (B), (C) and/or (D) is indicative of the endocrine disrupting activity of the test substance.

36. (Previously Presented) The method of Claim 29, wherein the RNA, or optionally the cDNA corresponding to said RNA, obtained from (A) and (B) and/or (C) and/or (D) is electrophoretically separated to determine the gene expression patterns.

37. (Previously Presented) The method of Claim 35, wherein the RNA, or optionally the cDNA corresponding to said RNA, obtained from (A) is hybridized to the RNA or cDNA obtained from (B) or (C) or (D), and the gene patterns of (A) and (B) or (C) or (D) are determined after subtraction of the hybridizing RNA or cDNA.

38. (Currently Amended) The method of Claim 29, wherein the gene expression pattern is determined by transcribed mRNA patterns, and wherein endocrine disruption is determined by identifying one or more types of mRNAs present in the gene expression pattern (1), but absent in at least one gene expression pattern selected from the group

consisting of gene expression patterns (2), (3) and (4), or identifying one or more mRNAs absent in the gene expression pattern (1), but present in at least one gene expression pattern selected from the group consisting of the gene expression patterns (2), (3) and (4) -expressed in (A), but not expressed in (B) or (C) or (D), or alternatively, one or more types of RNA expressed in (B) or (C) or (D), that are not expressed in (A).

39. (Previously Presented) The method of Claim 29, wherein endocrine disruption is determined by identifying that a different amount of one or more types of RNA is expressed in (A), compared to (B) or (C) or (D).

40. (Previously Presented) The method of Claim 29, comprising:

- (a) recovering RNAs from (A) and (B), and/or (C) and/or (D);
- (b) subjecting the RNAs recovered in step (a) to reverse transcription;
- (c) amplifying reverse transcription products obtained in (b) by PCR; and
- (d) subjecting PCR products obtained in step (c) to electrophoresis, comparing the electrophoretic patterns of bands obtained, thereby detecting a band specific to a first gene expression pattern of (A).

41. (Previously Presented) The method of Claim 29, wherein said gene expression patterns are measured by determining variation in the amount of protein or glycoprotein expression between (A) and (B) and/or (C) and/or (D).

42. (Previously Presented) The method of Claim 29, wherein one or more protein(s) or glycoprotein(s) expressed in (A) and (B) and/or (C) and/or (D) are electrophoretically separated to determine the gene expression patterns.

43. (Previously Presented) The method of Claim 42, wherein the protein(s) or glycoprotein(s) expressed in (A) and (B) and/or (C) and/or (D) are electrophoretically separated using SDS-PAGE to determine the respective gene expression patterns.

44. (Previously Presented) The method of Claim 42, wherein the protein(s) or glycoprotein(s) expressed in (A) and (B) and/or (C) and/or (D) are electrophoretically separated using two-dimensional electrophoresis to determine the respective gene expression patterns.

45. (Previously Presented) The method of Claim 29, wherein endocrine disruption is determined by identifying that a different amount of a protein or glycoprotein is expressed in the gene expression pattern of (A), compared to (B) or (C) or (D).

46. (Previously Presented) The method of Claim 29, comprising determining a variation in the amount of protein modification in said gene expression patterns, wherein a variation in protein modification between one or more proteins in the gene expression pattern of (A) compared to (B) and/or (C) and/or (D) is indicative of an endocrine disrupting activity of the test substance.

47. (Previously Presented) The method of Claim 46, where the variation in protein modification is measured by:

recovering the glycosylated proteins of (A), and (B) and/or (C) and/or (D) by binding them to a substance that binds to a polysaccharide chain,
cleaving the polysaccharide chain from the glycoprotein, and
determining the gene expression patterns obtained from (A) and (B) and/or (C) and/or (D) based on a comparison of the glycoproteins after cleavage.

48. (Previously Presented) The method of Claim 29, wherein said cell is a germ cell.

49. (Previously Presented) The method of Claim 29, wherein said cell is a nerve cell.

50. (Previously Presented) The method of Claim 29, wherein said cell is a normal cell.

51. (Previously Presented) The method of Claim 29, wherein said cell is a cancer cell.

52. (Previously Presented) The method of Claim 29, wherein said cell is a nonhuman mammalian cell.

53. (Previously Presented) The method of Claim 29, wherein said cell is a human cell.

54. (Previously Presented) The method of Claim 29, wherein said cell is not a genetically engineered cell.

55. (Previously Presented) The method of Claim 29, wherein said cell is selected from the group consisting of a murine neuroblastoma cell, a murine uterus carcinoma cell, a murine testicular Leydig cell, a cell derived from testicular Sertoli cells.

56. (Previously Presented) The method of Claim 29, wherein said cell is selected from the group consisting of Neuro2a, MCF7, TM3, TM4, 15P-1 and S-20Y.

57. (Previously Presented) The method of Claim 29, wherein said endocrine hormone is a female hormone.

58. (Previously Presented) The method of Claim 29, wherein said endocrine hormone is estrogen, estradiol, or progesterone.

59. (Previously Presented) The method of Claim 29, wherein said endocrine hormone is a male hormone.

60. (Previously Presented) The method of Claim 29, wherein said endocrine hormone is androgen, testosterone, or androsterone.

61. (Previously Presented) The method of Claim 29, wherein said endocrine hormone is an adrenal cortex hormone.

62. (Previously Presented) The method of Claim 29, wherein said endocrine hormone is cortisol, aldosterone, corticosterone or cortisone.

63. (Previously Presented) The method of Claim 29, wherein said endocrine hormone is an amino acid derivative hormone.

64. (Previously Presented) The method of Claim 29, wherein said endocrine hormone is triiodothyronine (T3), thyroxine (T4) or a parathyroid hormone.

65. (Previously Presented) A method of detecting an endocrine disrupting action of a test substance, comprising:

(A) culturing a cell, which has not been genetically engineered and which is sensitive to an endocrine hormone, in the presence of the endocrine hormone and the test substance and detecting a gene expression pattern (1) of said cell; and

(B) culturing said cell, which has not been genetically engineered and which is sensitive to an endocrine hormone, in the presence of the endocrine hormone, but in the absence of the test substance, and detecting a gene expression pattern (2) of said cell; and/or

(C) culturing said cell, which has not been genetically engineered and which is sensitive to an endocrine hormone, in the absence of the endocrine hormone, but in the presence of the test substance, and detecting a gene expression pattern (3) of said cell; and/or

(D) culturing said cell, which has not been genetically engineered and which is sensitive to an endocrine hormone, in the absence of the endocrine hormone and in the absence of the test substance, and detecting a gene expression pattern (4) of said cell; and

(E) comparing gene expression pattern (1) with gene expression pattern (2) and/or (3) and/or (4) to determine the endocrine disrupting activity of the test substance, wherein the increased or decreased expression of a gene in expression pattern (1) compared to the same gene in expression pattern (2) and/or (3) and/or (4) is indicative of endocrine disrupting action by the test substance.

66. (Previously Presented) A method of detecting an endocrine disrupting action of a test substance, comprising:

(A) culturing a cell, which has not been genetically engineered and which is sensitive to an endocrine hormone, in the presence of the endocrine hormone and the test substance and detecting a gene expression pattern (1) of said cell; and

(B) culturing said cell, which has not been genetically engineered and which is sensitive to an endocrine hormone, in the presence of the endocrine hormone, but in the absence of the test substance, and detecting a gene expression pattern (2) of said cell; and

(C) comparing gene expression pattern (1) with gene expression pattern (2) to determine the endocrine disrupting activity of the test substance, wherein the increased or decreased expression of a gene in expression pattern (1) compared to the same gene in expression pattern (2) is indicative of endocrine disrupting action by the test substance.

67. (Previously Presented) A method of detecting an endocrine disrupting action of a test substance, comprising:

(A) culturing a cell that is sensitive to an endocrine hormone in the presence of the endocrine hormone and the test substance and detecting a gene expression pattern (1) of said cell, and

(B) culturing said cell that is sensitive to an endocrine hormone in the presence of the endocrine hormone, but in the absence of the test substance, and detecting a gene expression pattern (2) of said cell; and

(C) comparing gene expression pattern (1) with gene expression pattern (2) to determine the endocrine disrupting activity of the test substance, wherein the increased or decreased expression of a gene in expression pattern (1) compared to the same gene in expression pattern (2) is indicative of endocrine disrupting action by the test substance.

68. (Currently Amended) The method of Claim 29, wherein gene expression pattern (1) is compared with gene expression pattern (2), ~~gene~~ gene expression pattern (3) and gene expression pattern (4).

69. (Previously Presented) The method of Claim 68, wherein gene expression pattern (1) is compared with gene expression pattern (2) to afford a comparison result, and then the comparison result is compared with the gene expression pattern (3) and gene expression pattern (4).

REMARKS/ARGUMENTS

Claims 29-69 are active. Claims 38 and 68 have been amended for clarity.

Accordingly, the Applicants do not believe that any new matter has been added. Endocrine disrupting substances, like dioxin, disrupt normal cellular homeostasis. Therefore, methods for detecting endocrine disrupting activity are of great importance. Unlike conventional methods for detecting substances which have endocrine disrupting activity, the presently claimed method is more sensitive in detecting compounds exerting endocrine disrupting activity because it detects endocrine disruption under conditions more similar to those found in a living organism, e.g., in the presence of an endocrine hormone. The superior sensitivity of the claimed method is shown in the prior Declaration, filed August 12, 2002.

The Applicants thank Examiner Chakrabarti for the courteous and helpful interview of June 3, 2003. As discussed, to address certain description or enablement concerns, the Applicants now file a Declaration under 37 C.F.R. 1.132 showing the RNA expression patterns produced under different control and experimental conditions. As also suggested, the Applicants provide herewith a copy of an ATCC catalog page indicating that cell line Neuro2A was established from a spontaneous tumor of strain A albino mice and is not indicated as a genetically engineered cell line by the ATCC. The Examiner agreed to consider the distinguishing features of the claimed comparative methods with respect to the prior art method of Lonial, upon which the anticipation and obviousness rejections depend.

Notwithstanding these distinguishing features, it was suggested that another way to address the outstanding prior art rejection would be to limit the claims to methods using nongenetically engineered cells. Claims 54, 65 and 66 are specifically directed to methods involving the use of non-genetically engineered cells. Accordingly, favorable consideration is now requested.

Rejection—35 U.S.C. 112, first paragraph

Claims 38, 54, 65 and 66 were rejected under 35 U.S.C. 112, first paragraph as lacking adequate description.

The Applicants submit that the rejection of Claim 38 may be withdrawn in view of the amendment of this claim. As requested to avoid a potential enablement concern with the claimed method, the Applicants now provide the attached Declaration under 37 C.F.R. 1.132, which shows a method for detecting endocrine disruption by determining differences in mRNA expression. Accordingly, the Applicants respectfully submit that this basis for rejection may be withdrawn.

With respect to Claims 65 and 66, the concern was that the limitation “which has not been genetically engineered” lacked descriptive support in the specification. That is, that this phrase did not have any basis in the original disclosure. The Applicants respectfully disagree, as page 11, lines 6-8, of the disclosure describes both normal cells, cancer cells and, alternatively, genetically engineered cells. Accordingly, a phrase excluding genetically engineered cells from the claimed method clearly has descriptive support in the original disclosure.

Rejection—35 U.S.C. 112, second paragraph

Claims 68 and 69 were rejected under 35 U.S.C. 112, second paragraph as being indefinite due to a typographical error. This rejection is moot in view of the correction of Claim 68.

Rejection—35 U.S.C. 102(e)

Claims 29-33, 46, 53 and 67 were rejected under 35 U.S.C. 102(e) as being anticipated by Lonial et al., U.S. Patent No. 6,001,560. The Applicants submit that this rejection should be withdrawn for the reasons of record and in view of the following:

Lonial is directed to a method of detecting IFN- γ antagonists, while the claimed method is directed to detecting endocrine disruption. The Official Action, page 19, indicated that this was considered an intended use and was therefore not accorded any patentable weight in distinguishing the claimed and prior art methods. The Applicants disagree and submit that this preamble breaths life and meaning into the recited method steps. For instance, the preamble of Claim 29 refers to a method of detecting an endocrine disrupting activity of a test substance, and the method steps in the body of the claim refer back to the preamble by requiring the selection of a cell sensitive to endocrine hormone, culturing the endocrine sensitive cell in the presence or absence of an endocrine hormone, and comparing gene expression patterns as an indication of endocrine disrupting activity. On the other hand, the Lonial method is specifically directed to detection of IFN- γ antagonists and does not contemplate the measurement of endocrine disruption.

Moreover, the method steps recited by the present claims are also distinguishable from the method steps disclosed by Lonial. Claims 65 and 66 require the use of a non-genetically engineered cell, and thus are distinguishable from the Lonial method which uses a genetically-engineered reporter cell.

The present claims, see e.g., Claim 29, also require the selection of an endocrine-sensitive cell, which is not suggested by Lonial. Claim 29 also requires that the endocrine sensitive cell be cultured in the presence of the endocrine hormone, which is also not suggested by Lonial (the growth hormone mentioned by Lonial is incidentally produced by a reporter gene). Importantly, the comparisons made by the Lonial method and the method of

the present invention are different. Lonial compares reporter gene expression of the cells contacted with an IFN- γ antagonist with the expression of cells not contacted with an IFN- γ antagonist. On the other hand, the present method compares the gene expression patterns of cells contacted with an endocrine hormone and a test substance with the gene expression patterns of (B) cells exposed to endocrine hormone, but not test substance, (C) cells exposed to test substance, but not endocrine hormone, or (D) cells not exposed to test substance or endocrine hormone. While Lonial compares reporter gene expression of cells exposed to a putative IFN- γ antagonist with reporter gene expression of control cells not exposed to the putative antagonists, this could not reasonably be considered a measurement of the endocrine disrupting activity of the test substance (putative IFN- γ antagonist). Finally, the Lonial method does not require a comparison for the purpose of determining endocrine disrupting activity. Accordingly, the Applicants respectfully request that this rejection be withdrawn.

Rejection—35 U.S.C. 103

Claims 37, 41, 42, 43, and 45 were rejected under 35 U.S.C. 103(a) as being anticipated by Lonial et al., U.S. Patent No. 6,001,560, in view of Gilles et al., U.S. Patent No. 4,663,281. This rejection may be withdrawn, as Lonial, as discussed above in the response to the anticipation rejection, does not disclose or suggest the claimed invention. Lonial, col. 2, lines 29-31 describes the search for IFN- γ agonists or antagonists, thus, there is no suggestion there to develop an assay system to detect endocrine disrupting compounds. Gilles, Figs. 2, 7 and 8 show the electrophoretic comparison of nucleic acids, but does not disclose or suggest the claimed method for detecting endocrine disrupting activity. Accordingly, the Applicants respectfully request that this rejection be withdrawn.

Rejection—35 U.S.C. 103

Claims 34, 35, 36, 39 and 40 were rejected under 35 U.S.C. 103(a) as being anticipated by Lonial et al., U.S. Patent No. 6,001,560, in view of Pearson et al., U.S. Patent No. 5,916,779. This rejection may be withdrawn, as Lonial, as discussed above in the response to the anticipation rejection, does not disclose or suggest the claimed invention. Pearson is cited as disclosing a method for recovering RNA and subjecting it to PCR to detect a band specific to a gene expression pattern, but does not disclose or suggest the claimed comparative method for detecting endocrine disrupting activity. Accordingly, the Applicants respectfully request that this rejection be withdrawn.

Rejection—35 U.S.C. 103

Claim 44 was rejected under 35 U.S.C. 103(a) as being anticipated by Lonial et al., U.S. Patent No. 6,001,560, in view of Schneider et al., U.S. Patent No. 6,537,432 B1. This rejection may be withdrawn, as Lonial, as discussed above in the response to the anticipation rejection, does not disclose or suggest the claimed invention. Schneider is cited as disclosing a method for electrophoretically separating proteins using 2D-electrophoresis, but does not disclose or suggest the claimed comparative method for detecting endocrine disrupting activity. Accordingly, the Applicants respectfully request that this rejection be withdrawn.

Rejection—35 U.S.C. 103

Claims 49, 55 and 56 were rejected under 35 U.S.C. 103(a) as being anticipated by Lonial et al., U.S. Patent No. 6,001,560, in view of Comoglio et al., U.S. Patent No. 6,030,949, and further in view of Cubicciotti, U.S. Patent No. 6,287,765 B1. This rejection may be withdrawn, as Lonial, as discussed above in the response to the anticipation rejection, does not disclose or suggest the claimed invention. Comoglio is cited as disclosing the cell

line Neuro 2A. Cubicciotti is not mentioned in the body of the rejection, though Gillies is. Gillies has been discussed above. Neither Comoglio, Cubicciotti nor Gillies disclose or suggest the claimed comparative method for detecting endocrine disrupting activity. Accordingly, the Applicants respectfully request that this rejection be withdrawn.

Rejection—35 U.S.C. 103

Claim 64 was rejected under 35 U.S.C. 103(a) as being anticipated by Lonial et al., U.S. Patent No. 6,001,560, in view of Cubicciotti, U.S. Patent No. 6,287,765 B1. This rejection may be withdrawn, as Lonial, as discussed above in the response to the anticipation rejection, does not disclose or suggest the claimed invention. Cubicciotti is cited as disclosing triiodothyronine, but does not disclose or suggest the claimed method for detecting endocrine disrupting activity using this hormone or cells sensitive to this hormone. Accordingly, the Applicants respectfully request that this rejection be withdrawn.

Rejection—35 U.S.C. 103

Claims 47 and 51 were rejected under 35 U.S.C. 103(a) as being anticipated by Lonial et al., U.S. Patent No. 6,001,560, in view of Makari U.S. Patent No. 4,752,471. This rejection may be withdrawn, as Lonial, as discussed above in the response to the anticipation rejection, does not disclose or suggest the claimed invention. Makari is cited as disclosing a method for removing a polysaccharide chain from a glycoprotein, but does not disclose or suggest the claimed method for detecting endocrine disrupting activity. Accordingly, the Applicants respectfully request that this rejection be withdrawn.

Rejection—35 U.S.C. 103

Claims 59-60 were rejected under 35 U.S.C. 103(a) as being anticipated by Lonial et al., U.S. Patent No. 6,001,560, in view of Soto U.S. Patent No. 5,135,849. This rejection may be withdrawn, as Lonial, as discussed above in the response to the anticipation rejection, does not disclose or suggest the claimed invention. Soto is cited as disclosing a male endocrine hormone and discloses methods for testing unevaluated substances for their androgen agonistic activities, but does not disclose or suggest the claimed comparative method for detecting endocrine disrupting activity using cells sensitive to a male endocrine hormone or a male endocrine hormone. Accordingly, the Applicants respectfully request that this rejection be withdrawn.

Rejection—35 U.S.C. 103

Claims 48, 50 and 52 were rejected under 35 U.S.C. 103(a) as being anticipated by Lonial et al., U.S. Patent No. 6,001,560, in view of Koretzky et al. U.S. Patent No. 6,194,633. This rejection may be withdrawn, as Lonial, as discussed above in the response to the anticipation rejection, does not disclose or suggest the claimed invention. Koretzky is cited as disclosing a method involving a germ cell and a normal nonhuman cell, but does not disclose or suggest the claimed comparative method for detecting endocrine disrupting activity. Accordingly, the Applicants respectfully request that this rejection be withdrawn.

Rejection—35 U.S.C. 103

Claims 48, 50 and 52 were rejected under 35 U.S.C. 103(a) as being anticipated by Lonial et al., U.S. Patent No. 6,001,560, in view of Firestone et al., U.S. Patent No. 6,150,395. This rejection may be withdrawn, as Lonial, as discussed above in the response to the anticipation rejection, does not disclose or suggest the claimed invention. Firestone is

cited as disclosing a female hormone estrogen, but does not disclose or suggest the claimed comparative method for detecting endocrine disrupting activity. Accordingly, the Applicants respectfully request that this rejection be withdrawn.

Rejection—35 U.S.C. 103

Claims 61-63 were rejected under 35 U.S.C. 103(a) as being anticipated by Lonial et al., U.S. Patent No. 6,001,560, in view of Mascarenhas, U.S. Patent No. 6,518,238 B1. This rejection may be withdrawn, as Lonial, as discussed above in the response to the anticipation rejection, does not disclose or suggest the claimed invention. Mascarenhas is cited as disclosing an endocrine hormone derived from cortisol, but does not disclose or suggest the claimed comparative method for detecting endocrine disrupting activity. Accordingly, the Applicants respectfully request that this rejection be withdrawn.

CONCLUSION

In view of the above amendments and remarks, the Applicants respectfully submit that this application is now in condition for allowance. Early notification to that effect is earnestly solicited.

Respectfully submitted,

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